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Morphine withdrawal syndrome: Involvement of the dopaminergic system in prepubertal male and female mice

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Abstract

Morphine (MOR) withdrawal signs are more marked in males than in females. Considering that the influence of the dopaminergic system on these differences is unclear, we analyzed dopamine (DA) and dihydroxyphenylacetic-acid (DOPAC) brain levels during naloxone (NAL)-precipitated withdrawal as well as the involvement of D_1 and D_2 receptors in the expression of MOR withdrawal in either sex. Prepubertal Swiss-Webster mice received MOR (2 mg/kg, i.p.) twice daily for 9 days. On the tenth day, dependent animals received NAL (6 mg/kg, i.p.) after MOR and were sacrificed 30 min later. DA and DOPAC concentrations were determined in different brain areas using HPLC with electrochemical detection. Other pool of mice received either a D_1 (SCH 23390; 0.2 mg/kg, i.p.) or D_2 (raclopride; 0.3 mg/kg, i.p.) receptor antagonist before NAL and withdrawal signs were evaluated. DA and DOPAC levels only decreased in striatum and cortex of withdrawn males. Conversely, both DA receptor antagonists decreased the expression of MOR withdrawal signs in either sex. The neurochemical sex differences described here could partially explain the behavioral sex differences observed during MOR withdrawal. Additionally, SCH-23390 and raclopride effects suggest an important role of both DA receptors in the expression of MOR withdrawal in males and females.

Keywords: Naloxone-precipitated withdrawal; Mice; Striatum; Frontal cortex; Dopamine; Sex differences; Raclopride; SCH 23390

1. Introduction

Several studies have demonstrated sex-related differences in many pharmacological properties of morphine (MOR): antinociception (Candido et al., 1992; Cicero et al., 1997), tolerance to analgesia (Kest et al., 2000) and conditioned analgesia (Stock et al., 2001). These studies stated that female rodents are less sensitive to MOR properties than males, but the mechanisms of this sex dimorphism still remain uncertain. In this context, we have demonstrated that female prepubertal mice were less prone to develop the signs of MOR withdrawal syndrome than males (Diaz et al., 2001) which is in agreement with previous results (Craft et al., 1999). Additionally, we have also demonstrated that an increase in μ-opioid receptor density occurred in male mice during the MOR withdrawal syndrome, but not in females (Diaz et al., 2004).

The development of opiate dependence as well as the expression of MOR withdrawal syndrome are both due to processes of homologous regulation affecting the endogenous opioid system and heterologous regulation that affect other neurotransmitter systems (Koob and Bloom, 1988). Previous studies have revealed that dopamine (DA) neurotransmission plays an important role in the MOR withdrawal syndrome (Acquas and Di Chiara, 1992; Diana et al., 1995; Diaz et al., 2003), but all these experiments have been performed in male animals, i.e., no data is available from females. In addition, there is a paucity of data about sex differences on brain monoamine concentrations during chronic MOR treatment and particularly, no information is available from mice. Brain areas such as striatum and frontal cortex have been related with the MOR withdrawal syndrome (Bassareo et al., 1995; Espejo et al., 2001; Diaz et al., 2003, 2004), but further studies would be necessary to explain the involvement of

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these areas in the expression of the MOR withdrawal syndrome.

In previous studies, DA agonists have been found able to potentiate several MOR withdrawal-induced signs in rodents (Gianutsos et al., 1976; Kantak and Miczek, 1988; Tidey and Miczek, 1992). Additionally, D_1 and D_2 receptor antagonists have been found to decrease the expression of MOR withdrawal-induced aggression in male mice (Rodriguez-Arias et al., 1999) as well as other signs in MOR withdrawn rats (Funada and Shippenberg, 1996; El-Kadi and Sharif, 1998; Zarrindast et al., 2002). Even though previous data suggest that the mesolimbic dopaminergic neurotransmission mediates several behavioral effects of opiates, the differential role of D_1 and D_2 receptors during MOR withdrawal remains unclear and has not been explored in female mice.

Since we have demonstrated sex-related behavioral differences in the expression of the MOR-withdrawal syndrome (Diaz et al., 2001) and taking into account the decreased striatal and cortical DA concentrations observed in MOR withdrawn male mice (Diaz et al., 2003), the aim of the present study was to analyze and compare striatal, cortical and hippocampal DA and dihydroxyphenylacetic acid (DOPAC) brain concentrations in prepubertal male and female mice during the MOR withdrawal syndrome. Another aim of this study was to evaluate the effect of the pretreatment of the selective D_1 (SCH 23390) and D_2 (raclopride) receptor antagonists during MOR withdrawal in either sex.

2. Methods

2.1. Subjects

Prepubertal Swiss-Webster male and female albino mice were obtained from our breeding colony of the Department of Pharmacology (Faculty of Pharmacy and Biochemistry) of the University of Buenos Aires. Experiments were performed on naïve prepubertal (indicated by vaginal smears) male and female mice weighing 20 g at the beginning of the treatment. Animals were housed in groups of five under conditions of constant temperature (22 ± 2 °C) and relative humidity (55 ± 15%), according to local regulations (SENASA). Mice were housed under a standard 12 h light/dark cycle (lights on 08:00 a.m.) with free access to food and water up to the beginning of the experiments. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs

Morphine hydrochloride (Chemotecnia Sintyal, Buenos Aires, Argentina), naloxone (Endo Laboratories, USA), SCH 23390 (RBI, USA) and raclopride (SIGMA-RBI, USA) were used to develop this study. All the drugs were dissolved in isotonic (NaCl 0.9%) saline solution and the doses were referred to the salt form. All the drugs were injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight.

2.3. Induction of MOR dependence

Mice were rendered dependent on MOR (as it was described in Diaz et al., 2001) by intraperitoneally (i.p.) injection of MOR (2 mg/kg), twice daily (10:00 h and 22:00 h), for 9 consecutive days.

2.4. Neurochemical studies

2.4.1. Drug treatment

On the day of the experiment (tenth day), dependent mice received the last dose of MOR only at 10:00 h and then were randomly divided into two groups ($n = 5–6$) as follows:

- **Dependence**: 60 min after the last dose of MOR, mice received saline (SAL).
- **MOR withdrawal**: 60 min after the last dose of MOR, mice received naloxone (NAL; 6 mg/kg). The dose of NAL used to precipitate the withdrawal syndrome in the present study was the same reported in our previous studies which did not induce a withdrawal syndrome in non-dependent mice, but did induce a full withdrawal syndrome in MOR-dependent mice (Diaz et al., 2001).

The control groups received SAL, twice daily for 9 consecutive days. On the tenth day animals received the last injection of SAL at 10:00 h and were divided into two control groups ($n = 5–6$):

- **SAL control**: 60 min after the last injection of SAL, mice received SAL.
- **NAL control**: 60 min after the last injection of SAL, mice received NAL.

2.4.2. Electrochemical detection

High Performance Liquid Cromatography (HPLC)-coupled electrochemical detection (Heikkila et al., 1984) of DA and DOPAC was achieved using a Varian 5000 liquid chromatograph coupled to an electrochemical detector (BAS LC-4C). Ten minutes after the last injection on the tenth day, brains were collected and striatum, cortex and hippocampus were dissected, weighed, homogenized, and deproteinized in 0.2 N perchloric acid (1/20 mg/ml). Homogenates were centrifuged and the supernatants were injected (50 μl) onto a 12.5 cm × 4 mm Nova-Pak C18 reverse phase column (Waters) developed in 250 ml of mobile phase (0.076 M $NaH_2PO_4 \cdot H_2O$; 5.24 ml/L PICB8, 0.99 mM EDTA, 6% methanol) 1.3 ml/min. The electrode potential was set at +0.7 V. Peak heights were measured by DATA Jet Integrator (Spectra-Physics) and quantified based on standard curves using Excel.

2.5. Behavioral studies

2.5.1. Drug treatment

The day of the experiment (tenth day), dependent mice received the last dose of MOR only at 10:00 h and